GB0404406

INVESTOR IN PEOPLE

The Patent Office
Concept House
Cardiff Road
Newport
South Wales
NP10 8QQ

PRIORITY DOCUMENT
SUBMITTED OR TRANSMITTED IN COMPLIANCE WITH RULE 17.1(a) OR (b)

I, the undersigned, being an officer duly authorised in accordance with Section 74(1) and (4) of the Deregulation & Contracting Out Act 1994, to sign and issue certificates on behalf of the Comptroller-General, hereby certify that annexed hereto is a true copy of the documents as originally filed in connection with the patent application identified therein.

In accordance with the Patents (Companies Re-registration) Rules 1982, if a company named in this certificate and any accompanying documents has re-registered under the Companies Act 1980 with the same name as that with which it was registered immediately before re-registration save for the substitution as, or inclusion as, the last part of the name of the words "public limited company" or their equivalents in Welsh, references to the name of the company in this certificate and any accompanying documents shall be treated as references to the name with which it is so re-registered.

In accordance with the rules, the words "public limited company" may be replaced by p.l.c., plc, P.L.C. or PLC.

Re-registration under the Companies Act does not constitute a new legal entity but merely subjects the company to certain additional company law rules.

Signed Stephen Hordley

Dated 8 November 2004

Patents Form 1/77

Patents Act 1977
(Rule 16)

Request for grant of a patent

(See the notes on the back of this form. You can also get an explanatory leaflet from the Patent Office to help you fill in this form)

The Patent Office

Cardiff Road
Newport
South Wales
NP10 8QQ

1. Your reference — P35684-/LMC/GST

2. Patent application number *(The Patent Office will fill in this part)* — 0324345.8

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

University Court of the University of Edinburgh
Old College
South Bridge
Edinburgh
EH8 9YL

Patents ADP number *(if you know it)* — 7986780001

If the applicant is a corporate body, give the country/state of its incorporation

4. Title of the invention — "Tissue Repair"

5. Name of your agent *(if you have one)*

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

Murgitroyd & Company
Scotland House
165-169 Scotland Street
Glasgow
G5 8PL

Patents ADP number *(if you know it)* — ~~1198013~~ 1198015

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number

Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application	Date of filing *(day / month / year)*

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:*

a) any applicant named in part 3 is not an inventor, or
b) there is an inventor who is not named as an applicant, or
c) any named applicant is a corporate body.
See note (d))

Yes

Patents Form 1/77

Patents Form 1/77

9. Enter the number of sheets for any of the following items you are filing with this form. Do not count copies of the same document

Continuation sheets of this form	
Description	45
Claim(s)	-
Abstract	-
Drawing(s)	8

10. If you are also filing any of the following, state how many against each item.

Priority documents	-
Translations of priority documents	-
Statement of inventorship and right to grant of a patent *(Patents Form 7/77)*	-
Request for preliminary examination and search *(Patents Form 9/77)*	-
Request for substantive examination *(Patents Form 10/77)*	-
Any other documents *(please specify)*	-

11. I/We request the grant of a patent on the basis of this application.

Signature Murgitroyd & Company Date 17 October 2002

Murgitroyd & Company

12. Name and daytime telephone number of person to contact in the United Kingdom — Gordon Stark — 0141 307 8400

Warning

After an application for a patent has been filed, the Comptroller of the Patent Office will consider whether publication or communication of the invention should be prohibited or restricted under Section 22 of the Patents Act 1977. You will be informed if it is necessary to prohibit or restrict your invention in this way. Furthermore, if you live in the United Kingdom, Section 23 of the Patents Act 1977 stops you from applying for a patent abroad without first getting written permission from the Patent Office unless an application has been filed at least 6 weeks beforehand in the United Kingdom for a patent for the same invention and either no direction prohibiting publication or communication has been given, or any such direction has been revoked.

Notes

a) *If you need help to fill in this form or you have any questions, please contact the Patent Office on 08459 500505.*

b) *Write your answers in capital letters using black ink or you may type them.*

c) *If there is not enough space for all the relevant details on any part of this form, please continue on a separate sheet of paper and write "see continuation sheet" in the relevant part(s). Any continuation sheet should be attached to this form.*

d) *If you have answered 'Yes' Patents Form 7/77 will need to be filed.*

e) *Once you have filled in the form you must remember to sign and date it.*

f) *For details of the fee and ways to pay please contact the Patent Office.*

I

"Tissue Repair"

The present invention relates to compounds and methods of repairing tissue in diseases where the extracellular matrix is degraded. More particularly the invention relates to compounds including antibodies which increase extracellular matrix anabolism and the use of a novel pathway to find compounds which are capable of use in therapy to increase extracellular matrix anabolism.

The Extracellular Matrix: Composition and Structure

The extracellular matrix (ECM) is a complex composite of proteins, glycoproteins and proteoglycans (PGs). Awareness of this complexity has been heightened by the recognition that ECM components, individually or

in concert with each other or other extracellular molecules, profoundly influence the biology of the cell and hence of the physiology of the whole structure in which the cell is embedded into. The functions of the ECM described so far are many but can be simply categorised as control of cell growth, providing structural support and physical stabilisation, affecting cell differentiation, orchestrating development and tuning metabolic responses (26).

PGs are a family of heterogeneous and genetically unrelated molecules. The number of full-time as well as part-time members is constantly expanding. The term full-time and part-time refers to the fact that some known PGs can exist as glycoproteins and some proteins can be found in a glycosylated form. In general, PGs are composed of a core protein to which one or more GAG chains are covalently attached by N or O linkage. GAGs are highly anionic linear heteropolysaccharides made of a disaccharide repeat sequences (36). However, there have been reports of PGs devoid of the GAG side chain (3,78). GAGs can be classified into four distinct categories based on their chemical composition (36). The first category is the chondroitin/dermatan sulphate (CS/DS) chain consisting of alternating galactosamine and glucuronic/iduronic acid units. A second class, which is by far the most structurally diverse, is the

heparin/heparan sulphate (H/HS) group which is composed of alternating glucosamine and glucuronic/iduronic repeats. The third type is the glucosamine and galactose containing keratan sulphate (KS) GAG. Hyaluronic acid (HA) is composed of glucosamine and glucuronic acid repeats. It is the most distinct GAG since it is not sulphated and is not covalently linked to the core protein of PG. Instead, HA binding to the PG core protein is mediated by a class of proteins known as HA binding proteins which exist in the ECM, on the cell surface and intracellularly (68).

Perlecan is a large HSPG with a core protein size of 400-450 kDa known to possess three HS chains. It was first isolated by Hassell et al. (28). It acquired its name from its appearance in rotary shadowing electron microscopy where it looks like a pearl on a string. It is a large multi-domain protein and thus one of the most complex gene products (16,35). Domain I is the N-terminus and contains acidic amino acid residues which facilitate the polymerisation of heparan sulphate (35). However, recombinant domain I has been shown to accept either HS or CS chains; an observation that has been confirmed by *in vitro* study characterising PGs synthesised in response to transforming growth factor β (TGF-β) and fetal calf serum showing that perlecan can be synthesised with CS chains (10). Ettner et al. (17) have shown that

the ECM glycoprotein laminin binds to perlecan domain I, as well as domain V both of which can carry the HS side chain. Loss of the HS chain abolished the binding.

Globular domain II was postulated to mediate ligand binding by the low-density lipoprotein (LDL) receptor due to their homology (21,53). Heparitinase treatment abrogates this interaction pointing to the fact that the HS GAG side chains are involved in the binding (21).

Domain III of perlecan contains an RGD tripeptide sequence that provides a binding capacity for integrin receptors and provides anchorage for the cell (13). Yamagata et al. have shown using double-immunofluorescence that perlecan colocalizes with integrins in cultured fibroblasts (76). This domain has also been shown to be homologous to the laminin short arm (34).

Domain IV is the largest domain of perlecan containing a series of Ig-like repeats similar to those found in the Ig superfamily of adhesion molecules leading to the speculation that it may function in intermolecular interactions (31). Finally, domain V possessing three globular domains homologous to the long arm of laminin is thought to

be responsible for self-assembly and laminin mediated cell adhesion (11).

The multiplicity and variety of perlecan's structural domains are indicative of its potential functions. Perlecan, in addition to binding to laminin and integrins, has been shown to bind fibronectin via its core protein (34). The HS chains of perlecan have also a very important functional role which has proven to be diverse. It has been reported that perlecan mediates the interaction between skeletal muscle cells and collagen IV via the HS GAG side chain (71). Recent studies have led to the identification and characterisation of perlecan as a ligand for L-selectin in the kidney (44). Whether this interaction is via the core protein and/or the HS side chain is not clear. The group of Varki has identified in a series of experiments the HS GAG as well as heparin from endothelial cells as a ligand for both L- and P- selectins but not E-selectins (40,54). The HS side chains in general, and those attached to perlecan core protein in particular, are known to bind growth factors such as fibroblast growth factors (FGF)-2, FGF-7, TGF-β, platelet factor-4 and platelet-derived growth factor-BB (PDGF-BB) (22,35). The functional significance of these interactions has been highlighted by numerous studies demonstrating the role of perlecan in angiogenesis (4,61), the control of smooth muscle cell growth (9)

and the maturation and maintenance of basement membranes (14). The functional importance of perlecan has been demonstrated by a study of mice lacking perlecan gene expression (14). Homozygous null mice died between embryonic days 10 and 12. The basement membranes normally subjected to increased mechanical stresses such as the myocardium lost their integrity and as a result small clefts formed in the cardiac muscle leading to bleeding in the pericardial sac and cardiac arrest. The homozygotes also had severe cartilage defects characterised by chondrodysplasia despite that fact that it is a tissue which normally lacks basement membrane. This finding was interpreted as a potential proteolysis-protective function for perlecan in cartilage (14). The delay in detecting abnormalities till E10 suggests a certain redundancy with compensatory molecules being able to substitute for perlecan such as the basement membrane HSPGs collagen XVIII (25) and agrin (24).

Large aggregating PGs are, to date, composed of four members; versican, aggrecan, neurocan and brevican (35). The hallmark of these PGs is the ability to bind hyaluronic acid forming highly hydrated aggregates. They are also characterised by their tridomain structure composed of an N-terminal domain where HA binding occurs, a central domain carrying the GAG side chains and lectin binding C-terminus.

Versican is a PG with a core protein of 265 - 370 kDa which was originally isolated from human fibroblasts and is the homologue of the avian PG-M (82). It can possess 10-30 chains of CS and has been also reported to carry KS GAG chains (81). It is expressed by keratinocytes, smooth muscle cells of the vessels, brain and mesengial cells of the kidney. The N-terminal domain is responsible for the hyaluronic acid binding properties of versican (42). The central domain of versican consists of the GAG binding subdomains, GAG-α and GAG-β. These subdomains are encoded by two alternatively spliced exons and this gives rise to different versican isoforms. To date four isoforms have been recognised. V0 contains both GAG-α and GAG-β. V1 and V2 are known to possess domain GAG-β and GAG-α respectively (81). V3 is the variant which contains neither of the two subdomains and hence carries no CS/DS GAG side chains and has been localised in various mammalian tissues (43,57,77). The third domain of versican is the C-terminus and consists of a lectin-binding domain, an EGF-like domain and a complement regulatory protein-like domain. This C-terminus binds the ECM glycoprotein, tenascin (2), heparin and heparan sulphate (62) and fibulin (1). Versican is known to have an inhibitory effect on mesenchymal chondrogenesis (80), promotes proliferation (79) and migration via the formation of pericellular matrices

via its interaction with cell surface bound hyaluronic acid (18). The formation of pericellular matrices is not only achieved via the core protein association with HA but also through GAG side chain interaction with the cytoskeletal associated cell surface receptor, CD44 (37). The postulated role of versican in migration has been also further reinforced by the recent findings of its interaction with both L- and P- selectins via the CS/DS side GAG chains (38). Furthermore, versican GAG side chains modulate chemokine response (30) and has been recently reported to possess growth factor binding capacity (83) and binding to β_1 integrin (75).

Aggrecan is another large aggrecating proteoglycan. It is known to be a major structural component of cartilage. It is composed of three globular domains and two GAG attachment domains (72). The N- terminal globular domain (G1) binds HA and link protein to form large aggregates. The second globular (G2) domain is unique to aggrecan and has no HA binding capacity. The function of this domain has not been clearly defined. The interglobular domain between the G1 and G2 contains proteolytic cleavage sites for metalloproteinases and thus been heavily investigated in pathologies where degradation of this domain is a hallmark, such as osteoarthritis. A KS domain is located at the C-terminus of the G2 domain followed by the CS domain. The CS domain is the largest domain

of aggrecan and the domain which contributes to the hydrated gel-like forming capacity of aggrecan and thus its importance in load-bearing function. The last domain is the globular domain (G3) which contains three modules: an epidermal growth factor-like domain, a lectin module and a complement regulatory module. This domain is responsible for the interaction of aggrecan with the ECM glycoprotein, tenascin.

Functions of Extracellular Matrix Proteoglycans

In addition to contributing to the mechanical properties of connective tissues, ECM PGs have biological functions which are achieved via specific classes of surface receptors. The two main, elaborately described, classes are syndecan and integrin receptor families (26). However, other receptors have also been described to bind ECM components such as the selecting family of glycoproteins (54), CD44 with all its variants (23), cell surface enzymes such as hyaluronic acid synthases (63), and PGs (35). It is important not to ignore the fact that the effects of the ECM do not and cannot, in an *in vivo* milieu, ever occur without the influence of other molecules. This statement is based on two well-described concepts. The first being that part of the effects of growth factors, cytokines, hormones and vitamins, as well as cell-to-

cell contact and physical forces is alteration of the ECM production. The second concept is that the effects of the ECM on the cell bear a striking similarity to those effects observed in response to the above mentioned factors. This is a phenomenon known as "mutual reciprocity" (26) which is an oversimplified view of a complex set of modular interactions, i.e. as defined by Hartwell et al. (27) "cellular functions carried out by "modules" made up of many species of interacting molecules". The outcome is a summation of all these modules which often interact with each other in a non-vectorial manner.

Integrins are a family of α,β heterodimeric receptors that mediate dynamic linkages between extracellular adhesion molecules and the intracellular actin cytoskeleton. Although integrins are expressed by all multicellular animals, their diversity varies widely among species (32,33,69). To date 19 α and 8 β subunit genes encode polypeptides that combine to form 25 different receptors. Integrins have been the subject of extensive research investigating the molecular and cellular basis of integrin function. Integrins are major contributors to both the maintenance of tissue integrity and the promotion of cellular migration. Integrin-ligand interactions provide physical support for cell cohesion, generation of traction forces in cellular movement, and organise signalling complexes

to modulate cellular functions such as differentiation and cell fate. PGs are key ECM components which interact with integrins modifying their function and integrins, in turn, are key regulators of ECM PGs. Furthermore, animal-model studies have also shown that integrins contribute to the progression of many common diseases, and thus are implicated as potential drugable targets. The use of anti-integrin monoclonal antibodies and ligand-mimetic peptides has validated this suggestion for inflammatory, neoplastic, traumatic and infectious conditions (15). This potential has lead to attempts to understand more about the mechanisms underlying tissue organisation and cellular trafficking, and to identify approaches for regulating these processes in disease, as well as determining the molecular basis of integrin function. To date the tertiary structure of the integrin dimer is unknown and therefore the current understanding of the molecular basis of integrin function is compiled from the results of a large number of studies which, although indirect, have employed a wide range of complementary technologies.

Maintenance of the Extracellular Matrix

ECM homeostasis is maintained under normal physiological conditions by a fine balance between degradation and synthesis orchestrated by matrix

metalloproteinase (MMPs) and tissue inhibitors of metalloproteinase (TIMPs). This homeostasis is critical in many physiological processes such as embryonic development, bone growth, nerve outgrowth, ovulation, uterine involution, and wound healing. MMPs also have a prominent role in pathological processes such as arthritis (45,49,60), chronic obstructive pulmonary disease (12,66) and atherosclerosis (46). Most of the research to data has focused on elucidating the regulation of these enzymes at the level of transcription, activation of proenzyme, and inhibition by natural inhibitors such as the tissue inhibitors of metalloproteinases. However, little is known about how they are anchored outside the cell.

Mechanical forces are known to modulate a variety of cell functions such as protein synthesis, proliferation, migration or survival and by doing so regulate tissue structure and function. The routes by which mechanical forces influence cell activities have been defined as mechanotransduction and include the tensegrity structure model and signalling through cell surface mechanoreceptors including ECM binding molecules. The tensegrity structure model postulates that a cell maintains a level of pre-stress generated actively by the actin microfilaments and intermediate filaments (48). This active stress element is balanced by structures resisting compression, mainly

microtubules within the cell and components of the ECM. Matrix remodelling in response to mechanical forces is an adaptive response to maintain tensegrity in mechanosensitive tissues including cartilage and lung. *In vivo* and *in vitro* observations demonstrate that mechanical stimulation is necessary to maintain optimal cartilage and lung structure and function (50,55,55,64,74). Thus mechanical forces regulate ECM composition which, in turn, will modify the mechanical microenvironment in tissues in a mutually reciprocal manner. This aspect provides a valuable tool for investigating biological functions in vitro.

Extracellular Matrix Catabolism and Anabolism

Cartilage is specialised connective tissue with a major function to bear mechanical stress without permanent distortion, acting as a shock absorber for joints. The ECM, accounting for 90% of the dry weight of cartilage consists primarily of large, hydrated PG aggregates trapped within a mesh of collagen fibrils. The ECM provides the tensile strength of cartilage as well as affecting cell behaviour.

Osteoarthritis (OA) is a pathological condition involving progressive degeneration of articular cartilage, remodelling of the sub-chondral bone accompanied by limited synovitis. Treatment of OA could be based on the emerging picture of

pathophysiological events that govern the initiation and progression of OA. The fundamental event leading to matrix destruction of articular cartilage in OA arises from an imbalance between anabolic and catabolic pathways (19,59). The ECM is degraded by matrix MMPs. Inhibition of the MMPs, their activators, and other factors that induce MMP gene up-regulation or activation is potentially a target for drug development in the treatment of OA (47). This is a novel modality of OA treatment which has focused in the past primarily on the administration of anti-inflammatory drugs such as corticosteroids and non-steroidal anti-inflammatory drugs (NSAIDs) which provide the patient with symptomatic relief from pain and swelling of synovial joints without affecting disease progression (i.e. cartilage degradation and osteophyte formation). Therefore, the statement of Hunter that, "ulcerated cartilage is a troublesome thing; once destroyed is not repaired" remains true to this day (47).

Novel therapeutic approaches include the application of tissue engineering to the repair of articular surface defects in OA or after injury which will require that viable cells be transplanted to the injured tissue site. This remains a challenging task since the long-term durability of this treatment approach remains in doubt. Genetic engineering offers another therapeutic approach to the regeneration of

degenerative articular cartilage. The objective is to control the biological systems which are mediating the destruction of ECM in OA. One of the major impediments in this area, however, is which genes to use and how and for how long can they be transferred safely (29,47,65,67).

Another organ system involving ECM is the lung where it provides structural support and acts as adhesive as well as a guiding cue for diverse biological processes. Collagens are the most abundant ECM component in the lung constituting 60-70 % of lung interstitium followed by elastin and PGs and glycoproteins (70).

Chronic Obstructive Pulmonary Disease (COPD) is another disease in whose progression ECM plays a major role. COPD is a major cause of chronic morbidity and mortality throughout the world. According to the latest statistics by the World Health Organisation (WHO), COPD is the fourth leading cause of death in the world, and further increases in its prevalence and mortality are predicted in the coming decades. Recently a Global Initiative for Chronic Obstructive Lung Disease (GOLD) was established in collaboration with the US National Heart, Lung, and Blood Institute (NHLBI) and the WHO. According to GOLD, "COPD is a disease state characterised by airflow limitation that is not fully

reversible. The airflow limitation is usually both progressive and associated with an abnormal inflammatory response of the lungs to noxious particles or gases". The major symptoms of COPD are cough, sputum production, and dyspnea upon exertion. Of these symptoms, the first to arise is chronic cough and sputum production followed by the development of airflow limitation many years later which could identify individuals at risk of developing COPD. COPD can coexist with asthma, the other major chronic obstructive airway disease characterised by an underlying airway inflammation. However, the inflammation characteristic of COPD is thought to be distinct from that of asthma and the airflow limitation in asthma is far more reversible than that featured in COPD.

Although the main risk factor for COPD is tobacco smoking, other predisposing factors have been identified. The main cause of chronic airflow limitation in COPD is small airway disease (obstructive bronchiolitis) and parenchymal destruction (emphysema). Chronic inflammation leads to small airway remodelling and subsequent narrowing. Destruction of the lung parenchyma, also by inflammatory processes, leads to the loss of alveolar attachments to the small airways and decreases lung elastic recoil. Based on mechanical interdependence, the loss of alveolar attachment also contributes to

the diminished ability of the airways to remain open during expiration. Thus, the major processes known to play a key role in the pathogenesis of COPD are inflammation, an imbalance of proteinases and antiproteinases in the lung, and oxidative stress which leads to the destruction of the ECM (56).

To date, the only available drug treatments for COPD sufferers focus primarily on bronchodilation using anticholinergics and dual β2-dopamine$_2$ receptor antagonists. Since the inflammation in COPD is resistant to corticosteroids, much anticipation is put into novel anti-inflammatory agents currently in development which include phosphodiesterase inhibitors, nuclear factor κB inhibitors and p38 MAP kinase inhibitors. Matrix metalloproteinase (MMP) inhibitors are also currently being developed although in their current formulation they have serious toxicity side effects. However, what is lacking so far is an agent which may aid in the repair of injured ECM (6-8).

In summary, both disease paradigms have a strong element of ECM remodelling as a major contributor in their pathophysiology. Furthermore, current therapeutics have focused primarily on preventative or symptom-relieving treatments. However, due to the progressive nature of both diseases together with

often late diagnosis, regaining normal function remains a problem.

Recently, novel therapeutic approaches targeting integrin function have been adopted. Very late antigen-4 (VLA4) or α4 integrin antagonists are currently in advance stages of trials for the treatment of asthma (39,41,51). In cartilage, antagonists to αvβ3 integrin have attenuated adjuvant-induced arthritis and now are undergoing trials (5). However, in both cases the target of the functional blocking is attenuating inflammation and this has not been demonstrated to affect the ECM alteration usually associated with those conditions.

Accordingly, it is an object of the present invention to provide a compound for use in ECM anabolism. It is a further aim of the present invention to provide a technique to screen compounds for use in ECM anabolism.

According to the present invention there is provided a compound for use in tissue repair wherein the compound modifies the function of β1 integrin.

Modification includes a change in the function of , or the inhibition of the binding of, or the shedding of the β1 integrin.

Preferably the compound is an inhibitor of the β1 integrin.

More preferably the compound functionally blocks β1 integrin.

Most preferably the compound binds the molecule in the region of amino acid residues 82-87 of β1 integrin.

In the known sequence, residues 82 to 87 are considered to be the residues of the sequence nprgsk (Asparagine-Proline-Arginine-Glycine-Serine-Lysine).

In one embodiment the compound is a synthetic peptide.

In a further embodiment the compound is an antibody.

The antibody should preferably be a humanised antibody.

Alternatively the antibody could be a chimeric antibody.

In one embodiment the antibody could be based on or derived from the functional modifying antibody of β1 integrin obtainable as a commercial clone JB1a from Chemicon.

The term antibody describes an immunoglobulin (Ig) molecule which is naturally synthesised or alternatively which is partly or wholly synthetically produced. Further, the term also includes any natural protein or partly or wholly synthetically produced protein having a binding domain which is, or is substantially homologous to an immunoglobulin binding site.

The invention also provides a method to screen for compounds for use in tissue repair wherein the method comprises the step of determining the ability of a compound to modify the function of the β1 integrin.

Modification includes a change in the function of , or the inhibition of the binding of, or the shedding of the β1 integrin.

Preferably the method comprises the step of determining the ability of a compound to bind the domain corresponding to residues 82-87 of β1 integrin (residues nprgsk (Asparagine-Proline-Arginine-Glycine-Serine-Lysine)).

The invention further provides a compound identified from the method described herein.

The invention further provides a medicament suitable for use in tissue repair wherein the medicament includes as an active ingredient, a compound which modifies the function of β1 integrin. Preferably the compound binds amino acid residues 82-87 of β1 integrin (residues nprgsk (Asparagine-Proline-Arginine-Glycine-Serine-Lysine)).

The medicament may be administered together with any suitable carrier.

The invention further provides the use of an antibody to β1 integrin in the preparation of a medicament for treatment of injured tissue.

The invention further provides the use of the sequence of amino acids at positions 82-87 of β1 integrin in the identification of a compound to promote tissue repair by increasing ECM anabolism.

The sequence of amino acids 82-87 is nprgsk (Asparagine-Proline-Arginine-Glycine-Serine-Lysine).

In a yet further embodiment of the present invention, there is provided a compound which causes the shedding of the β1 integrin.

Preferably the compound binds amino acid residues 82-87 of β1 integrin.

In the known sequence, residues 82-87 are considered to be the residues of the sequence nprgsk (Asparagine-Proline-Arginine-Glycine-Serine-Lysine).

In one embodiment the compound is a synthetic peptide.

In a further embodiment the compound is an antibody.

The antibody should preferably be a humanised antibody.

Alternatively the antibody could be a chimeric antibody.

It is thought that a compound according to the present invention may act by shedding the β1 integrin.

Substitutions may be made to the binding epitope as defined in the present invention, for example amino acid residues may be substituted with a residues of the same or similar chemical class, and which result in no substantial conformational change of the binding epitope.

In a yet further embodiment of the present invention, there is provided a compound for use in tissue repair wherein the compound binds to any nature-similar or mimetic molecule which has conformational homology to the β1 integrin. In other words the three-dimensional shape of the mimetic molecule is substantially super-imposable upon the three-dimensional shape of the β1 integrin.

Preferably the nature-similar or mimetic molecule has a conformational homology to amino acid residues 82 to 87 of the β1 integrin.

Residues 82 to 87 are known to have the sequence nprgsk (Asparagine-Proline-Arginine-Glycine-Serine-Lysine).

In a preliminary experiment, the present inventors attempted to investigate the role of the cell surface receptors in the synthesis of ECM which are altered in diseases such as COPD and OA and are important for lung and cartilage function microscopically and macroscopically.

The results described herein demonstrate that functional modification of β1 integrin through a domain corresponding to amino acid residues 82-87 induces a substantial time- and dose- dependent increase in ECM in normal, osteoarthritic and lung

epithelial cells in monolayer and 3-dimensional cultures. The 3-dimensional alginate culture prevents dedifferentiation of chondrocytes and hence the loss of chondrocytic phenotype. This inhibitory domain is different from those previously described which bind to amino acid sequence with residues 207-218 (20). It is also distinct from the known stimulatory domains which are localised to those amino acid residues and residues 657-670 and 671-703 (73). Modulation of the cytokine TGF-β induced a less profound increase which was also time- and dose-dependent. This increase in all ECM PGs was sustained for extended periods of time without any additive doses.

These experiments demonstrate a novel finding which is that an increase in ECM can be achieved via the modulation of cell surface receptors and to a much lesser extent by modulating the binding of a soluble factor in a time- and dose-dependent manner in both pulmonary and cartilage derived cells. When performed in conjunction with mechanical stimulation, the ECM increase demonstrated altered organisation which could signify functionality of the PGs. One potential, but non-binding mechanistic hypothesis is that this modulation may have led to alteration in the proteinase/antiproteinase balance which can be sequestered onto the surface of cells since the response was so rapid that no gene transcription or translation could have occurred.

The potential of these findings lie in tissue repair in disease where the matrix is degraded and cannot be replenished as in diseases like COPD and OA. The finding may offer a venue for therapeutic intervention in such diseases where the only current lines of therapy focus on alleviating the symptoms by the use of anti-inflammatory agents but has no potential for regaining function. This could be achieved via the administration of humanised or chimeric antibodies or synthetic peptides capable of binding the specific region of the β1 integrin. In summary, the results herein address a different potential therapeutic modality which focuses on increasing ECM anabolism instead of decreasing catabolism.

The invention is exemplified herein with reference to the following non limiting examples and with reference to the figures.

In the figures:

Figure 1 illustrates time- and dose-dependent effects of functional modifying antibody of β1 integrin and TGF-β on proteoglycans,

Figure 2 illustrates the effect of β1 integrin functional modification in combination with mechanical stimulation of monolayer cultures,

Figure 3 illustrates the effect of β1 integrin and TGFB blocking in combination with mechanical stimulation of normal chondrocytes,

Figure 4 illustrates the effect of β1 integrin functional modification and TGF-β blocking in combination with mechanical stimulation on osteoarthritic chondrocytes,

Figure 5 illustrates PG content in the alginate cultures in response to functional modification of β1 integrin and neutralising TGF-β in relation to time and dose,

Figure 6 illustrates dose- and time-dependent effects of functional modification of β1 integrin and neutralising TGF-β on ECM PG from H441 cell lines, and

Figure 7 shows the amino acid sequence of β1 integrin which is nprgsk (Asparagine-Proline-Arginine-Glycine-Serine-Lysine), and

Figure 8 shows the presence of a 110kDa β1 integrin in the media of chondrocytes in alginate cultures and H441 cells separated onto 6% SDS-polyacrylamide gels following β1 integrin function modulation but not following TGF-β neutralisation.

Stimulation Protocol and Extraction Procedure

Primary human chondrocytes from articular knee joints were obtained with consent and cultured in either monolayers ($5x10^4$/ml) or embedded into 1% alginate (1:1 v/v of Keltone and Pronova) at density of $5x10^4$ and $50x10^4$ /ml respectively in Iscoves media (Sigma Aldrich) and maintained at 5% in an CO_2 incubator. Cultures were subjected to serum starving overnight in a medium containing 0.5% foetal calf serum. Functional modifying antibody of β1 integrin (Chemicon, clone JB1a) was added to the cultures at concentration of 1.44 and 0.48 µg/ml. The β1 integrin stimulatory antibody TS2/16 which binds the amino acid region 207-218 was also added at 0.9 µg/ml for 1 hour to demonstrate the specificity of the JB1a action. TGFβ neutralising antibody (R&D systems, clone 1D11) was added at a concentration of 0.1 and 0.3 µg/ml where at the lower concentration it neutralises TGFβ isoforms 1 and 3 and isoform 2 at the higher concentration. After antibody addition to

the cells in culture, the medium was aspirated and the cell layer rinsed twice with ice-cold PBS (calcium- and magnesium-free). The media was aspirated and preserved after the addition of protease inhibitors at -80°C. PGs were extracted from the cell layer by extraction buffer containing protease inhibitors (4M guanidium-HCl, 4% (w/v) CHAPS, 100mM sodium acetate buffer at pH 5.8 containing protease inhibitors) for 24 hours at 4°C. Monolayers were subjected to mechanical stimulation 4000μ strain at 0.33 Hz for 20 min after the addition of the antibodies (30 min) or PBS and collected for extraction after one hour.

Adenocarcinoma cell line derived from the lung were also used (H441) to test the effect of the antibodies on matrix synthesis. This cell line has epithelial type II characteristics.

The total protein concentration was estimated using the Bradford method.

Sample Preparation for Composite Polyacrylamide-Agarose Gel Electrophoresis

The extracts were precipitated overnight with 9 v/v ethanol at -20°C, centrifuged at 12,000 rpm for 40min at 4°C then resuspended in 0.5M sodium acetate (pH 7.3) and precipitated again with ethanol overnight

and centrifuged. Samples were resuspended in 0.5% SDS and mixed with 1:1 v/v with 50%w/w sucrose in 10mM Tris-HCl (pH 6.8), 0.5% SDS and 0.05% bromophenol blue. 20ug of protein was used for gel loading.

Gel electrophoresis

Composite gels (1.5mm thick) containing 0.6% agarose and 1.2% polyacrylamide in Tris-sodium acetate buffer (10mM, pH 6.8) containing 0.25mM sodium sulphate were used for the separation of large PG, versican, aggrecan and perlecan, under associative conditions according to the method of Carney.

After electrophoretic separation, the samples were transferred onto Hybond ECL-nitrocellulose membrane (Amersham Pharmacia) using a wet blotting unit (BioRad). Membranes were blocked with 5% Milk in TBS pH 7.4 containing 0.1 % v/v Tween-20 and 0.1% sodium azide for 1 hours at room temperature and then incubated with primary antibodies diluted in TBS-Tween 20 for 1 hour at room temperature or overnight at 4°C. The primary antibody for versican (12C5) was mouse anti-human at 1/500 dilution (Hybridoma Bank, Iowa City, Iowa). This antibody recognises the hyaluronic acid binding domain of versican (58). Aggrecan antibody was used at dilution of 1/500 aggrecan (Serotec, HAG7E1). Due to the fact that the exact epitope recognised by this antibody is unknown,

additional antibodies were used. An antibody recognising amino acid sequence 1-14 in the hyaluronic acid region was used at 1/500 dilution (Biogenesis, Germany). The anti-interglobular domain region antibody 6B4 was also used to confirm the identity of aggrecan (gift from Prof. Bruce Caterson, Cardiff). Perlcan antibody was use at a dilution of 1/1000 (7B5, Zymed Laboratories). This antibody has been demonstrated to be immunoreactive to non-degraded forms of perlecan (52). A biotinylated secondary antibody (goat anti mouse Ig, Dako) was added, followed by a 1 hour incubation with 1/1000 streptavidin-horseradish peroxidase (HRP) complex in TBS-T. Signal was visualised using the ECLplus (enhanced chemiluminescence) assay (Amersham Pharmacia).

The same analyses as detailed above were performed using extracts subjected to pre-clearing of the functional modifying antibodies by immunoprecipitation using protein A sepharose according to manufacturer's instructions (Amersham Pharmacia).

Our experiments demonstrate a novel finding which is that the an increase in ECM PGs anabolism can be achieved via functional modification of the cell surface β1 integrin and to a much lesser extent to neutralising TGFβ in both time- and dose-dependent

manner in primary chondrocytes derived from normal and osteoarthritic articular cartilage as well as pulmonary derived cells. This response is also achieved in non-dedifferentiated chondrocyte cultures maintained in alginate matrix. One potential mechanistic hypothesis is that the functional modification of integrin may have led to increased release of tissue inhibitors of MMP (TIMP2) which are known to be sequestered onto the surface of cells by various cell surface molecules such as membrane type-1 MMP. TIMP2 is an inhibitor of MMP2. MMP2 could lead to matrix PG degradation either directly or via inducing MMP9. Thus modifying integrin could have led to inactivation of MMP2 and subsequently MMP9 leading to increased matrix PG accumulation. However, this could be one mechanism and the involvement of another pathway could not be excluded. As to the less profound effect of neutralising TGFβ, the answer could lie in the known interaction of MMP9 with TGFβ where it has been shown that MMP9 can activate latent TGFβ, rendering it biologically active.

Although the present invention has been particularly shown and described with reference to a particular example, it will be understood by those skilled in the art that various changes in the form and details may be made therein without departing from the scope of the present invention.

References

1. Aspberg, A., S. Adam, G. Kostka, R. Timpl, and D. Heinegard. Fibulin-1 is a ligand for the C-type lectin domains of aggrecan and versican. *J.Biol.Chem.* 274: 20444-20449, 1999.

2. Aspberg, A., R. Miura, S. Bourdoulous, M. Shimonaka, D. Heinegard, M. Schachner, E. Ruoslahti, and Y. Yamaguchi. The C-type lectin domains of lecticans, a family of aggregating chondroitin sulfate proteoglycans, bind tenascin-R by protein-protein interactions independent of carbohydrate moiety. *Proc.Natl.Acad.Sci.U.S.A* 94: 10116-10121, 1997.

3. Aumailley, M. and B. Gayraud. Structure and biological activity of the extracellular matrix. *J.Mol.Med.* 76: 253-265, 1998.

4. Aviezer, D., D. Hecht, M. Safran, M. Eisinger, G. David, and A. Yayon. Perlecan, basal lamina proteoglycan, promotes basic fibroblast growth factor-receptor binding, mitogenesis, and angiogenesis. *Cell* 79: 1005-1013, 1994.

5. Badger, A. M., S. Blake, R. Kapadia, S. Sarkar, J. Levin, B. A. Swift, S. J. Hoffman, G. B. Stroup, W. H. Miller, M. Gowen, and M. W. Lark. Disease-modifying activity of SB 273005, an orally active, nonpeptide alphavbeta3 (vitronectin receptor) antagonist, in rat

adjuvant-induced arthritis. *Arthritis Rheum.* 44: 128-137, 2001.

6. Barnes, P. J. Future Advances in COPD Therapy. *Respiration* 68: 441-448, 2001.

7. Barnes, P. J. New treatments for chronic obstructive pulmonary disease. *Curr.Opin.Pharmacol.* 1: 217-222, 2001.

8. Barnes, P. J. Potential novel therapies for chronic obstructive pulmonary disease. *Novartis.Found.Symp.* 234: 255-267, 2001.

9. Bingley, J. A., I. P. Hayward, J. H. Campbell, and G. R. Campbell. Arterial heparan sulfate proteoglycans inhibit vascular smooth muscle cell proliferation and phenotype change in vitro and neointimal formation in vivo. *J.Vasc.Surg.* 28: 308-318, 1998.

10. Brown, C. T., M. A. Nugent, F. W. Lau, and V. Trinkaus-Randall. Characterization of proteoglycans synthesized by cultured corneal fibroblasts in response to transforming growth factor beta and fetal calf serum. *J.Biol.Chem.* 274: 7111-7119, 1999.

11. Brown, J. C., T. Sasaki, W. Gohring, Y. Yamada, and R. Timpl. The C-terminal domain V of perlecan promotes beta1 integrin-mediated cell adhesion, binds heparin, nidogen and fibulin-2 and can be modified by glycosaminoglycans. *Eur.J.Biochem.* 250: 39-46, 1997.

12. Cawston, T., S. Carrere, J. Catterall, R. Duggleby, S. Elliott, B. Shingleton, and A. Rowan. Matrix metalloproteinases and TIMPs: properties and implications for the treatment of chronic obstructive pulmonary disease. *Novartis.Found.Symp.* 234: 205-218, 2001.

13. Chakravarti, S., T. Horchar, B. Jefferson, G. W. Laurie, and J. R. Hassell. Recombinant domain III of perlecan promotes cell attachment through its RGDS sequence. *J.Biol.Chem.* 270: 404-409, 1995.

14. Costell, M., E. Gustafsson, A. Aszodi, M. Morgelin, W. Bloch, E. Hunziker, K. Addicks, R. Timpl, and R. Fassler. Perlecan maintains the integrity of cartilage and some basement membranes. *J.Cell Biol.* 147: 1109-1122, 1999.

15. Curley, G. P., H. Blum, and M. J. Humphries. Integrin antagonists. *Cell Mol.Life Sci.* 56: 427-441, 1999.

16. Dunlevy, J. R. and J. R. Hassell. Heparan Sulfate Proteoglycans in Basement Membranes: Perlecan, Agrin, and Collagen XVIII. In Iozzo, R. V., ed. Proteoglycans: Structure, Biology and Molecular Interactions. New York, Marcel Dekker. 2000, 275-326.

17. Ettner, N., W. Gohring, T. Sasaki, K. Mann, and R. Timpl. The N-terminal globular domain of the laminin alpha1 chain binds to alpha1beta1 and alpha2beta1 integrins and to the heparan

sulfate- containing domains of perlecan. *FEBS Lett.* 430: 217-221, 1998.

18. Evanko, S. P., J. C. Angello, and T. N. Wight. Formation of hyaluronan- and versican-rich pericellular matrix is required for proliferation and migration of vascular smooth muscle cells. *Arterioscler.Thromb.Vasc.Biol.* 19: 1004-1013, 1999.

19. Felson, D. T., R. C. Lawrence, P. A. Dieppe, R. Hirsch, C. G. Helmick, J. M. Jordan, R. S. Kington, N. E. Lane, M. C. Nevitt, Y. Zhang, M. Sowers, T. McAlindon, T. D. Spector, A. R. Poole, S. Z. Yanovski, G. Ateshian, L. Sharma, J. A. Buckwalter, K. D. Brandt, and J. F. Fries. Osteoarthritis: new insights. Part 1: the disease and its risk factors. *Ann.Intern.Med.* 133: 635-646, 2000.

20. Fernandez, C., K. Clark, L. Burrows, N. R. Schofield, and M. J. Humphries. Regulation of the extracellular ligand binding activity of integrins. *Front Biosci.* 3: D684-D700, 1998.

21. Fuki, I. V., R. V. Iozzo, and K. J. Williams. Perlecan heparan sulfate proteoglycan: a novel receptor that mediates a distinct pathway for ligand catabolism. *J.Biol.Chem.* 275: 25742-25750, 2000.

22. Gallagher, J. T. Structure-activity relationship of heparan sulphate. *Biochem.Soc.Trans.* 25: 1206-1209, 1997.

23. Goodison, S., V. Urquidi, and D. Tarin. CD44 cell adhesion molecules. *Mol.Pathol.* 52: 189-196, 1999.

24. Groffen, A. J., C. A. Buskens, T. H. van Kuppevelt, J. H. Veerkamp, L. A. Monnens, and L. P. van den Heuvel. Primary structure and high expression of human agrin in basement membranes of adult lung and kidney. *Eur.J.Biochem.* 254: 123-128, 1998.

25. Halfter, W., S. Dong, B. Schurer, and G. J. Cole. Collagen XVIII is a basement membrane heparan sulfate proteoglycan. *J.Biol.Chem.* 273: 25404-25412, 1998.

26. Haralson, M. A. and J. R. Hassell. The extracellular matrix- an overview. In Haralson, M. A. and J. R. Hassell, eds. Extracellular Matrix: A practical approach. Oxford University Press. 1995, 1-30.

27. Hartwell, L. H., J. J. Hopfield, S. Leibler, and A. W. Murray. From molecular to modular cell biology. *Nature* 402: C47-C52, 1999.

28. Hassell, J. R., P. G. Robey, H. J. Barrach, J. Wilczek, S. I. Rennard, and G. R. Martin. Isolation of a heparan sulfate-containing proteoglycan from basement membrane. *Proc.Natl.Acad.Sci.U.S.A* 77: 4494-4498, 1980.

29. Hinton, R., R. L. Moody, A. W. Davis, and S. F. Thomas. Osteoarthritis: diagnosis and

therapeutic considerations. *Am.Fam.Physician* 65: 841-848, 2002.

30. Hirose, J., H. Kawashima, O. Yoshie, K. Tashiro, and M. Miyasaka. Versican interacts with chemokines and modulates cellular responses. *J.Biol.Chem.* 2000.

31. Hopf, M., W. Gohring, E. Kohfeldt, Y. Yamada, and R. Timpl. Recombinant domain IV of perlecan binds to nidogens, laminin-nidogen complex, fibronectin, fibulin-2 and heparin. *Eur.J.Biochem.* 259: 917-925, 1999.

32. Humphries, M. J. Integrin cell adhesion receptors and the concept of agonism. *Trends Pharmacol.Sci.* 21: 29-32, 2000.

33. Humphries, M. J. Integrin structure. *Biochem.Soc.Trans.* 28: 311-339, 2000.

34. Iozzo, R. V. Perlecan: a gem of a proteoglycan. *Matrix Biol.* 14: 203-208, 1994.

35. Iozzo, R. V. Matrix proteoglycans: from molecular design to cellular function. *Annu.Rev.Biochem.* 67: 609-652, 1998.

36. Jackson, R. L., S. J. Busch, and A. D. Cardin. Glycosaminoglycans: molecular properties, protein interactions, and role in physiological processes. *Physiol Rev.* 71: 481-539, 1991.

37. Kawashima, H., M. Hirose, J. Hirose, D. Nagakubo, A. H. Plaas, and M. Miyasaka. Binding of a large chondroitin Sulfate/Dermatan sulfate proteoglycan, versican, to L-selectin, P-

selectin, and CD44. *J.Biol.Chem.* 275: 35448-35456, 2000.

38. Kawashima, H., Y. F. Li, N. Watanabe, J. Hirose, M. Hirose, and M. Miyasaka. Identification and characterization of ligands for L-selectin in the kidney. I. Versican, a large chondroitin sulfate proteoglycan, is a ligand for L-selectin. *Int.Immunol.* 11: 393-405, 1999.

39. Knight, D. Epithelium-fibroblast interactions in response to airway inflammation. *Immunol.Cell Biol.* 79: 160-164, 2001.

40. Koenig, A., K. Norgard-Sumnicht, R. Linhardt, and A. Varki. Differential interactions of heparin and heparan sulfate glycosaminoglycans with the selectins. Implications for the use of unfractionated and low molecular weight heparins as therapeutic agents. *J.Clin.Invest* 101: 877-889, 1998.

41. Kraneveld, A. D., A. van, I, H. J. Van Der Linde, D. Fattah, F. P. Nijkamp, and A. J. Van Oosterhout. Antibody to very late activation antigen 4 prevents interleukin-5- induced airway hyperresponsiveness and eosinophil infiltration in the airways of guinea pigs. *J.Allergy Clin.Immunol.* 100: 242-250, 1997.

42. Lebaron, R. G., D. R. Zimmermann, and E. Ruoslahti. Hyaluronate binding properties of versican. *J.Biol.Chem.* 267: 10003-10010, 1992.

43. Lemire, J. M., K. R. Braun, P. Maurel, E. D. Kaplan, S. M. Schwartz, and T. N. Wight. Versican/PG-M isoforms in vascular smooth muscle cells. *Arterioscler.Thromb.Vasc.Biol.* 19: 1630-1639, 1999.

44. Li, Y. F., H. Kawashima, N. Watanabe, and M. Miyasaka. Identification and characterization of ligands for L-selectin in the kidney. II. Expression of chondroitin sulfate and heparan sulfate proteoglycans reactive with L-selectin. *FEBS Lett.* 444: 201-205, 1999.

45. Little, C. B., C. E. Hughes, C. L. Curtis, M. J. Janusz, R. Bohne, S. Wang-Weigand, Y. O. Taiwo, P. G. Mitchell, I. G. Otterness, C. R. Flannery, and B. Caterson. Matrix metalloproteinases are involved in C-terminal and interglobular domain processing of cartilage aggrecan in late stage cartilage degradation. *Matrix Biol.* 21: 271-288, 2002.

46. Loftus, I. M., A. R. Naylor, P. R. Bell, and M. M. Thompson. Matrix metalloproteinases and atherosclerotic plaque instability. *Br.J.Surg.* 89: 680-694, 2002.

47. Malemud, C. J. and V. M. Goldberg. Future directions for research and treatment of osteoarthritis. *Front Biosci.* 4: D762-D771, 1999.

48. Maniotis, A. J., C. S. Chen, and D. E. Ingber. Demonstration of mechanical connections between

integrins, cytoskeletal filaments, and nucleoplasm that stabilize nuclear structure. *Proc.Natl.Acad.Sci.U.S.A* 94: 849-854, 1997.

49. Mengshol, J. A., K. S. Mix, and C. E. Brinckerhoff. Matrix metalloproteinases as therapeutic targets in arthritic diseases: bull's-eye or missing the mark? *Arthritis Rheum.* 46: 13-20, 2002.

50. Millward-Sadler, S. J., M. O. Wright, L. W. Davies, G. Nuki, and D. M. Salter. Mechanotransduction via integrins and interleukin-4 results in altered aggrecan and matrix metalloproteinase 3 gene expression in normal, but not osteoarthritic, human articular chondrocytes. *Arthritis Rheum.* 43: 2091-2099, 2000.

51. Milne, A. A. and P. J. Piper. Role of the VLA-4 integrin in leucocyte recruitment and bronchial hyperresponsiveness in the guinea-pig. *Eur.J.Pharmacol.* 282: 243-249, 1995.

52. Murdoch, A. D., B. Liu, R. Schwarting, R. S. Tuan, and R. V. Iozzo. Widespread expression of perlecan proteoglycan in basement membranes and extracellular matrices of human tissues as detected by a novel monoclonal antibody against domain III and by in situ hybridization. *J.Histochem.Cytochem.* 42: 239-249, 1994.

53. Noonan, D. M., A. Fulle, P. Valente, S. Cai, E. Horigan, M. Sasaki, Y. Yamada, and J. R.

Hassell. The complete sequence of perlecan, a basement membrane heparan sulfate proteoglycan, reveals extensive similarity with laminin A chain, low density lipoprotein-receptor, and the neural cell adhesion molecule. *J.Biol.Chem.* 266: 22939-22947, 1991.

54. Norgard-Sumnicht, K. and A. Varki. Endothelial heparan sulfate proteoglycans that bind to L-selectin have glucosamine residues with unsubstituted amino groups. *J.Biol.Chem.* 270: 12012-12024, 1995.

55. Otterness, I. G., J. D. Eskra, M. L. Bliven, A. K. Shay, J. P. Pelletier, and A. J. Milici. Exercise protects against articular cartilage degeneration in the hamster. *Arthritis Rheum.* 41: 2068-2076, 1998.

56. Parks, W. C. and S. D. Shapiro. Matrix metalloproteinases in lung biology. *Respir.Res.* 2: 10-19, 2001.

57. Paulus, W., I. Baur, M. T. Dours-Zimmermann, and D. R. Zimmermann. Differential expression of versican isoforms in brain tumors. *J.Neuropathol.Exp.Neurol.* 55: 528-533, 1996.

58. Perides, G., F. Rahemtulla, W. S. Lane, R. A. Asher, and A. Bignami. Isolation of a large aggregating proteoglycan from human brain. *J.Biol.Chem.* 267: 23883-23887, 1992.

59. Poole, A. R. An introduction to the pathophysiology of osteoarthritis. *Front Biosci.* 4: D662-D670, 1999.

60. Poole, A. R., G. Rizkalla, M. Ionescu, A. Reiner, E. Brooks, C. Rorabeck, R. Bourne, and E. Bogoch. Osteoarthritis in the human knee: a dynamic process of cartilage matrix degradation, synthesis and reorganization. *Agents Actions Suppl* 39: 3-13, 1993.

61. Sharma, B., M. Handler, I. Eichstetter, J. M. Whitelock, M. A. Nugent, and R. V. Iozzo. Antisense targeting of perlecan blocks tumor growth and angiogenesis in vivo. *J.Clin.Invest* 102: 1599-1608, 1998.

62. Shinomura, T., Y. Nishida, K. Ito, and K. Kimata. cDNA cloning of PG-M, a large chondroitin sulfate proteoglycan expressed during chondrogenesis in chick limb buds. Alternative spliced multiforms of PG-M and their relationships to versican. *J.Biol.Chem.* 268: 14461-14469, 1993.

63. Spicer, A. P. and McDonald, J. A. Eukaryotic Hyaluronan Synthases. http://www.glycoforum.gr.jp/science/hyaluronan/HA07/HA07E.html.1999. Seikagaku Japan.

64. Takahashi, I., G. H. Nuckolls, K. Takahashi, O. Tanaka, I. Semba, R. Dashner, L. Shum, and H. C. Slavkin. Compressive force promotes sox9, type II collagen and aggrecan and inhibits IL-1beta

expression resulting in chondrogenesis in mouse embryonic limb bud mesenchymal cells. *J.Cell Sci.* 111 (Pt 14): 2067-2076, 1998.

65. Terchek, M., J. Baker, B. Goeckeritz, and D. Loebl. Osteoarthritis--a review. *J.Med.Assoc.Ga* 91: 22-26, 2002.

66. Thickett, D. R., A. R. Poole, and A. B. Millar. The balance between collagen synthesis and degradation in diffuse lung disease. *Sarcoidosis.Vasc.Diffuse.Lung Dis.* 18: 27-33, 2001.

67. Todd, C. Meeting the therapeutic challenge of the patient with osteoarthritis. *J.Am.Pharm.Assoc.(Wash.)* 42: 74-82, 2002.

68. Toole, B. P. Hyaluronan and its binding proteins, the hyaladherins. *Curr.Opin.Cell Biol.* 2: 839-844, 1990.

69. Tuckwell, D. S. and M. J. Humphries. Molecular and cellular biology of integrins. *Crit Rev.Oncol.Hematol.* 15: 149-171, 1993.

70. Turino, G. M. The lung parenchyma--a dynamic matrix. J. Burns Amberson lecture. *Am.Rev.Respir.Dis.* 132: 1324-1334, 1985.

71. Villar, M. J., J. R. Hassell, and E. Brandan. Interaction of skeletal muscle cells with collagen type IV is mediated by perlecan associated with the cell surface. *J.Cell Biochem.* 75: 665-674, 1999.

72. Watanabe, H., Y. Yamada, and K. Kimata. Roles of aggrecan, a large chondroitin sulfate proteoglycan, in cartilage structure and function. *J.Biochem.(Tokyo)* 124: 687-693, 1998.

73. Wilkins, J. A., A. Li, H. Ni, D. G. Stupack, and C. Shen. Control of beta1 integrin function. Localization of stimulatory epitopes. *J.Biol.Chem.* 271: 3046-3051, 1996.

74. Wirtz, H. R. and L. G. Dobbs. The effects of mechanical forces on lung functions. *Respir.Physiol* 119: 1-17, 2000.

75. Wu, Y., L. Chen, P. S. Zheng, and B. B. Yang. beta 1-Integrin-mediated glioma cell adhesion and free radical-induced apoptosis are regulated by binding to a C-terminal domain of PG-M/versican. *J.Biol.Chem.* 277: 12294-12301, 2002.

76. Yamagata, M., S. Saga, M. Kato, M. Bernfield, and K. Kimata. Selective distributions of proteoglycans and their ligands in pericellular matrix of cultured fibroblasts. Implications for their roles in cell-substratum adhesion. *J.Cell Sci.* 106 (Pt 1): 55-65, 1993.

77. Zako, M., T. Shinomura, and K. Kimata. Alternative splicing of the unique "PLUS" domain of chicken PG- M/versican is developmentally regulated. *J.Biol.Chem.* 272: 9325-9331, 1997.

78. Zako, M., T. Shinomura, M. Ujita, K. Ito, and K. Kimata. Expression of PG-M(V3), an alternatively spliced form of PG-M without a chondroitin

sulfate attachment in region in mouse and human tissues. *J.Biol.Chem.* 270: 3914-3918, 1995.

79. Zhang, Y., L. Cao, C. Kiani, B. L. Yang, W. Hu, and B. B. Yang. Promotion of chondrocyte proliferation by versican mediated by G1 domain and EGF-like motifs. *J.Cell Biochem.* 73: 445-457, 1999.

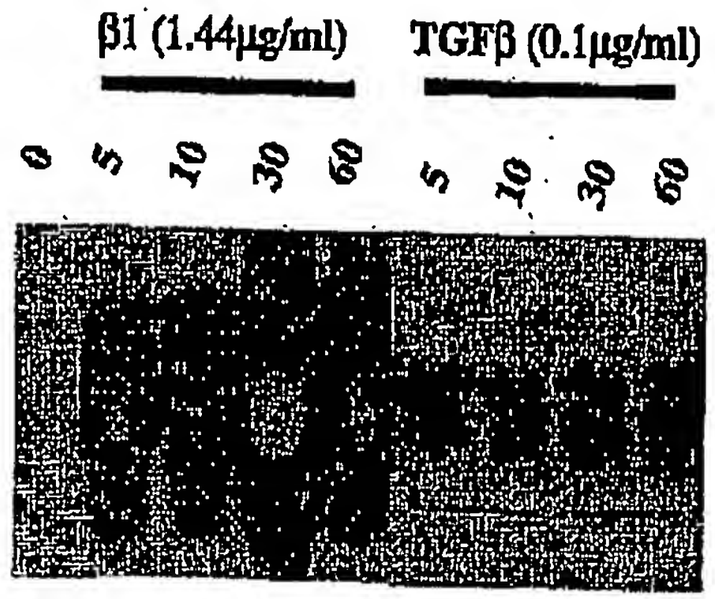
80. Zhang, Y., L. Cao, B. L. Yang, and B. B. Yang. The G3 domain of versican enhances cell proliferation via epidermial growth factor-like motifs. *J.Biol.Chem.* 273: 21342-21351, 1998.

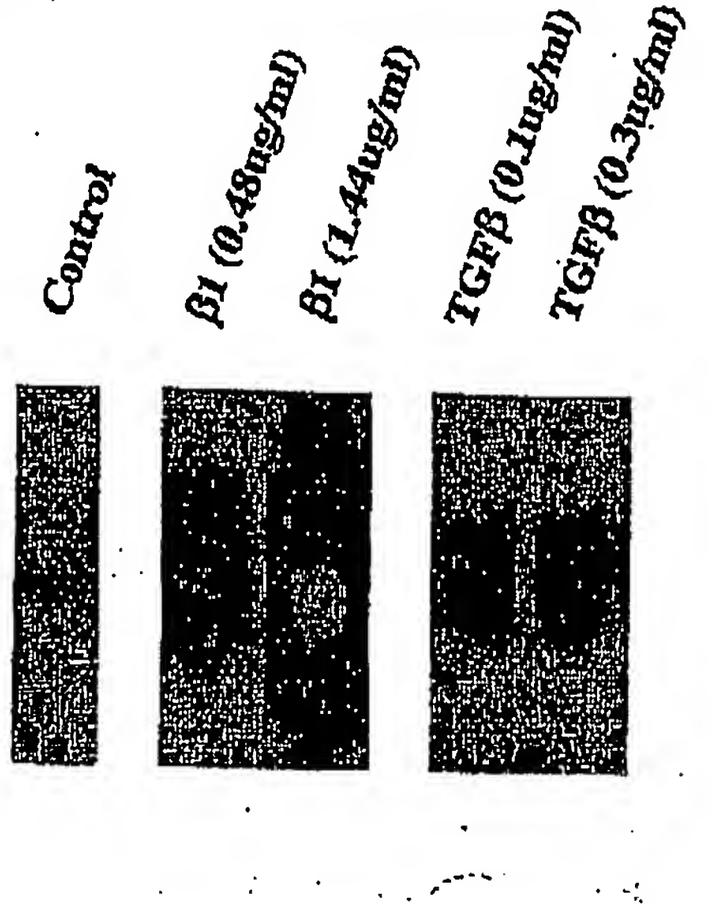
81. Zimmermann, D. R. Versican . In Iozzo, R. V., ed. Proteoglycans: Structure, Biology and Molecular Interactions. New York, Marcel Dekker 2000, 327-342.

82. Zimmermann, D. R. and E. Ruoslahti. Multiple domains of the large fibroblast proteoglycan, versican. *EMBO J.* 8: 2975-2981, 1989.

83. Zou, K., H. Muramatsu, S. Ikematsu, S. Sakuma, R. H. Salama, T. Shinomura, K. Kimata, and T. Muramatsu. A heparin-binding growth factor, midkine, binds to a chondroitin sulfate proteoglycan, PG-M/versican. *Eur.J.Biochem.* 267: 4046-4053, 2000.

Figure 1.





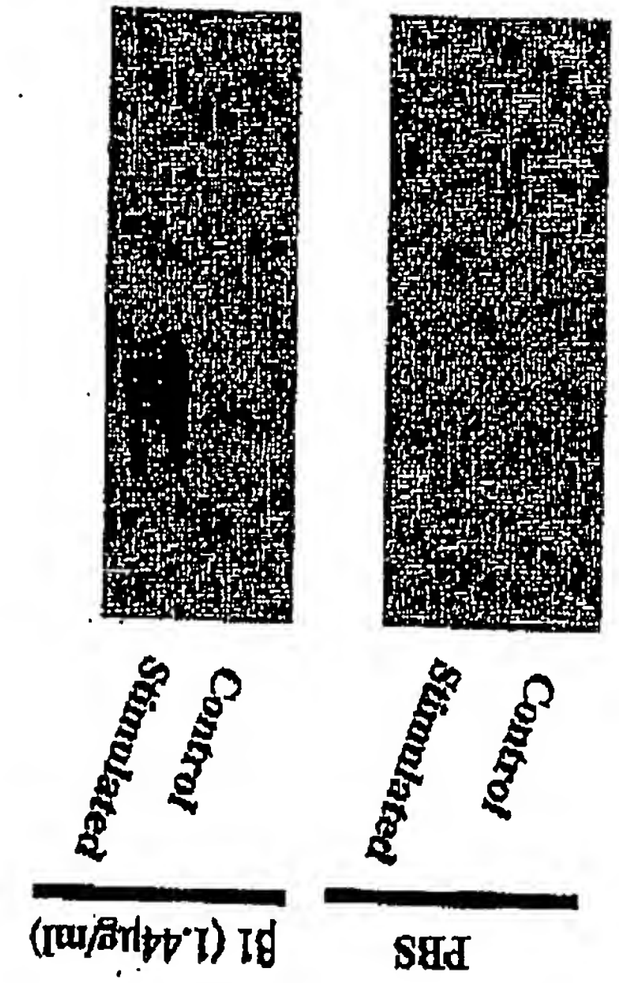


Figure 2.

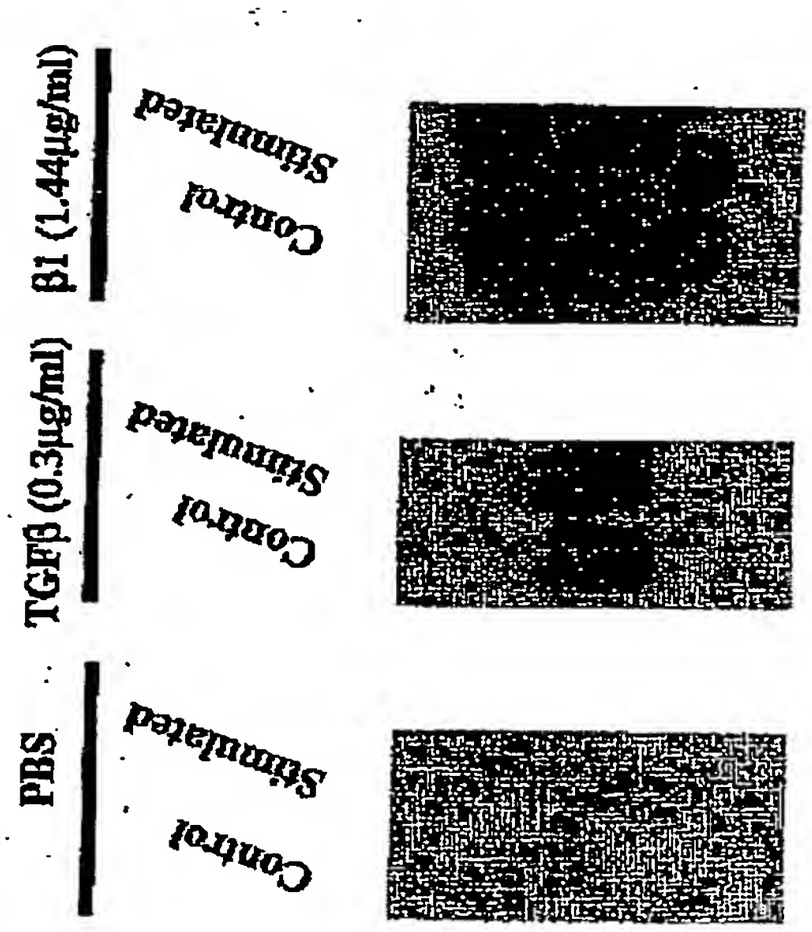

PBS
Control
Stimulated
TGFβ (0.3µg/ml)
Control
Stimulated
β1 (1.44µg/ml)
Control
Stimulated

Figure 3.

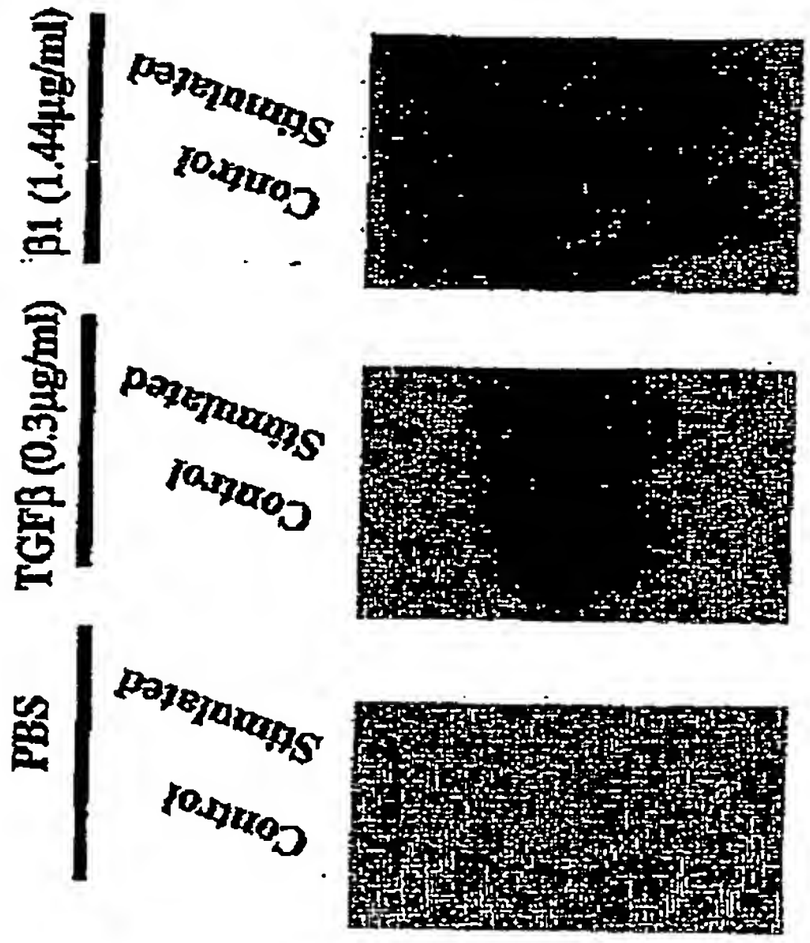

PBS
Control
Stimulated
TGFβ (0.3μg/ml)
Control
Stimulated
β1 (1.44μg/ml)
Control
Stimulated

Figure 4.

Figure 5.

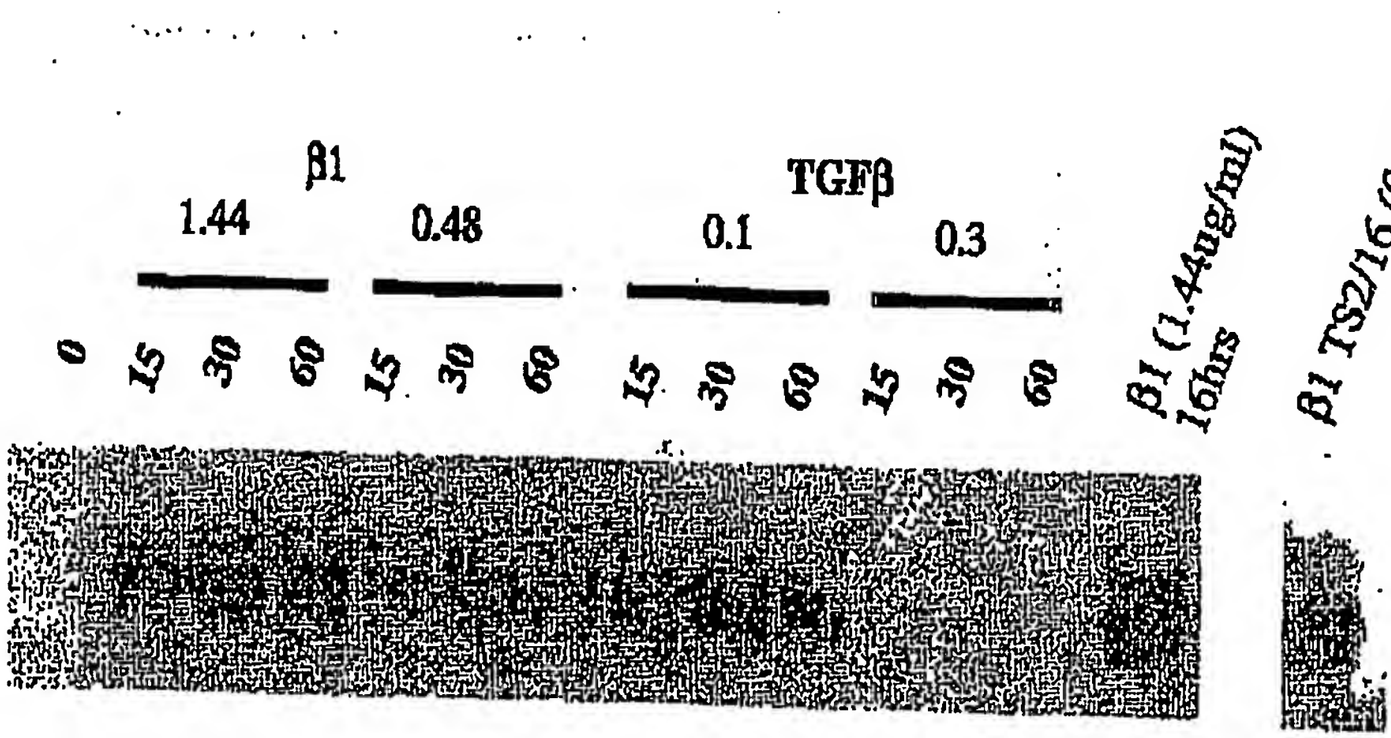


Figure 6.

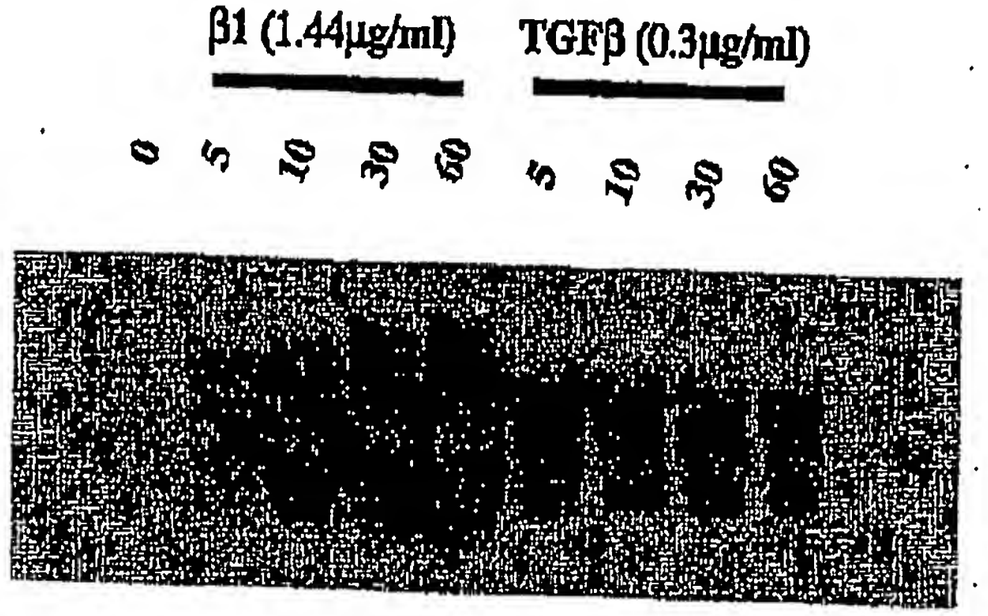


Figure 7. - Amino Acid Sequence of beta - 1 integrin

MNLQPIFWIGLISSVCCVFAQTDENRCLKANAKSCGECIQAGPNC

GWCTNSTFLQEGMPTSARCDDLEALKKKGCPPDDIENPRGSKDIKKNKNVTNRSKGTAE

KLKPEDIHQIQPQQLVLRLRSGEPQTFTLKFKRAEDYPIDLYYLMDLSYSMKDDLENVK

SLGTDLMNEMRRITSDFRIGFGSFVEKTVMPYISTTPAKLRNPCTSEQNCTTPFSYKNV

LSLTNKGEVFNELVGKQRISGNLDSPEGGFDAIMQVAVCGSLIGWRNVTRLLVFSTDAG

FHFAGDGKLGGIVLPNDGQCHLENNMYTMSHYYDYPSIAHLVQKLSENNIQTIFAVTEE

FQPVYKELKNLIPKSAVGTLSANSSNVIQLIIDAYNSLSSEVILENGKLSEGVTISYKS

YCKNGVNGTGENGRKCSNISIGDEVQFEISITSNKCPKKDSDSFKIRPLGFTEEVEVIL

QYICECECQSEGIPESPKCHEGNGTFECGACRCNEGRVGRHCECSTDEVNSEDMDAYCR

KENSSEICSNNGECVCGQCVCRKRDNTNEIYSGKFCECDNFNCDRSNGLICGGNGVCKC

RVCECNPNYTGSACDCSLDTSTCEASNGQICNGRGICECGVCKCTDPKFQGQTCEMCQT

CLGVCAEHKECVQCRAFNKGEKKDTCTQECSYFNITKVESRDKLPQPVQPDPVSHCKEK

DVDDCWFYFTYSVNGNNEVMVHVVENPECPTGPDIIPIVAGVVAGIVLIGLALLLIWKL

LMIIHDRREFAKFEKEKMNAKWDTGENPIYKSAVTTVVNPKYEGK

Figure 8.